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Diploma Thesis

Osmolality of electrolyte infusions

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Statement of originality

I declare that this diploma thesis is my own personal work and that I worked on it on my own. All literature and other resources that I used, are listed in the reference list and are properly cited.

Date: Signature:

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1 Abstract

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The aim of this thesis is to study the osmotic properties of model infusion solutions of sodium chloride and potassium chloride. In a concentration range of 0.01 – 1,00mol/kg, density at 20°C and osmolality is measured. The average values of density at 20°C and/or osmolality are used to convert molality to molarity and osmolality to osmolarity, respectively. The average partial specific volume and the average molal volume of the dissolved solute 0.29 ml/g and 16.80 ml/molwas estimated experimentally for sodium chloride. For potassium chloride, the average volumes are 0.36 ml/g and 26.84 ml/mol, respectively.

2 The aim of study

In the theoretical section, the aim of this thesis is to present the general information about the parenteral preparations, their formulation and quality requirements. Particularly, the osmotic properties of infusions are studied.

In the experimental part, the aqueous solutions of sodium chloride and potassium chloride in a concentration range of 0.01 – 1,00mol/kg are prepared and their density and osmolality is measured. The results are used to convert molality to molarity as well as osmolality to osmolarity, to estimate the partial specific volume and the molal volume of the dissolved solute, and, finally, to estimate the molal osmotic coefficient.

3 List of abbreviations

Symbol	Unit	Meaning
c_{os}	mOsmol/l	Osmolarity
m_{os}	mOsmol/kg	Osmolality
c	mol/l	Molarity
m	mol/kg	Molality
M_0	g	Mass of solute
M	g	Mass of solution
d	g/ml	Density
V_w	ml	Water volume
V_g	ml/g	Partial specific volume
V_{mol}	ml/mol	Molal volume
C	g/ml	Concentration
Φ_m	-	Molal osmotic coefficient

4 Introduction

Parenteral preparations are dosage forms of drugs that are injected directly to body tissue usually through the skin or mucous membranes, in different routes of administration. Since the drugs are directly injected to the body tissue they must be pure, isotonic, free from various types of contaminants, and compatible with other substances that might be needed to be applied together. Administration of parenteral dosage forms might be required for various reasons such as patient incompliance or inactivation of the active substance in the gastrointestinal tract (GIT), although they might have some disadvantages. That being said, production of parenteral preparations is a big responsibility and their administration requires caution and control (Troy, 2005).

The aim of this thesis is to study the methods that are being used for the whole production process (including sterilization and shelf-life) of large volume parenteral products, according to their type and use, and focus on electrolyte solutions and their properties. Also the importance of the osmotic properties (especially of osmotic pressure) to the human body is going to be expressed. And finally, in the last part of this document are going to be displayed in graphs the relationships between molality and some other important values such as: partial specific volume, molal volume, and molal osmotic coefficient; the conversion of osmolality to osmolarity is also going to be explained.

5 Theoretical section

5.1 Parenteral Preparations

There are some general factors that need to be taken into consideration when developing a parenteral drug. Those factors determine the drug dosage form and its formulation. Such factors are: the route of administration, the pharmacokinetics of the drug, the compatibility of the drug with other "co-substances" when administered together, and the drug solubility and stability.

The route of administration may vary and includes many different ways. Parenteral drugs can be injected different ways such as subcutaneously, intramuscularly, intravenously, intra-arterially, intrathecally, and intradermally. The dosage form of the drug usually determines its route of administration (i.e only microheterogenousemulsions o/w or solutions can be administered intravenously).

The pharmacokinetics of the drug also determine the dosage form as well as the route of administration. The rates at which the drug is being absorbed, distributed, metabolised and excreted, can be modified in order to become more rapid or retarded. It should be mentioned that these rules do not apply for intravenous or intra-arterial routes of administration.

The compatibility of the drug with other "co-substances" is very important. It is possible that an excipient added to the formulation of the parenteral preparation will help the drug's action in one way and at the same time create a problem at the drug's action in a different way. For example the added "co-substance" may stabilize chemically the drug and at the same time catalyse some other reactions, that degrade the drug (i.e chemical degradation or oxidative degradation).

Last but not least, **drug solubility and drug stability** must be taken into consideration when developing the formulation of a parenteral preparation. Solubility indicates the appropriate concentration of the drug in the dosage form. In the case where the drug is not soluble enough in a specific dosage, then substances that increase the drug's solubility need to be added. Sterile solid dosage forms need to be

created if the drug appears stability issues. Stability is also related to the expiration date of the drug andalso to its storage conditions (Troy, 2005).

5.1.1 Excipients in small volume parenterals (SVPs)

In order to achieve safety and efficacy of parenteral products many times various substances should be added into the formula to maintain pharmaceutical stability of the product, ensure its sterility or to aid in parenteral administration. Such substances are antioxidants, buffers, bulking materials, chelating agents, inert gases, solubilizing agents and protectants. In such preparations it is important to choose added substances that are accepted by regulatory agencies throughout the world.

As **antioxidants** in aqueous parenterals, thesubstances that are most frequently used are salts of sulphur dioxide, including bisulphite, meta-sulphite and sulphite.These manage to maintain the stability of the drugs by being preferentially oxidised and gradually consumed over the shelf life of the product. It is possible that sulphites cause allergic reactions in some asthmatics. In this case it would be better to consider alternative use of antioxidants or to manufacture and pack the product in such a way in order to eliminate or to minimize the concentration of bisulphite required.

Many times a certain pH is required to maintain the stability of the drug and also solubility is strongly dependent on the pH of the solution. In order to achieve and maintain specific pH values, **buffers** are added into the solutions and that is why SVPs should be formulated in such way in order to have sufficient buffer capacity. Buffers for parenterals usually consist of either a weak base and the salt of a weak base or a weak acid and the salt of a weak acid. Last but not least, factors that influence the pH value of the product are the product degradation, diffusion of gases (through the closure), and container and stopper effects.

Chelating agents are also used to inactivate metals that generally catalyse oxidative degradation of drug molecules. Sources of metal (copper, iron, zinc) contamination

include raw material impurities, solvents (i.e. water), manufacturing equipment and rubber stoppers and containers.
Another way of protecting the oxygen-sensitive medicaments is by displacing the air in the solution with nitrogen or argon. Reducing the dissolved oxygen by boiling the water or by purging with nitrogen makes this technique more effective.

Surfactants and **solubilizing agents** are also used as excipients in small volume parenterals. Solubilizing agents are added to increase drug solubility and it is recommended that their effect on the safety and stability of the drug, must be considered. Surfactants are surface active agents that are used to disperse a water-insoluble drug as a colloidal dispersion. They prevent crystals from growing in the suspension and they are also used for wetting of powders in order to provide acceptable syringability.

Other important excipients that are added in injectable solutions, especially small volume preparations, are **tonicity adjustment agents**. It is very important for such preparations (intravenous) to be isotonic. It is a fact that the osmotic pressure changes and as a result, an exchange of ionic species across red blood cell membranes, non-isotonic solutions occurs. Especially if given in volumes larger than 100 ml, hypotonic or hypertonic solutions can lead to hemolysis or crenation of red blood cells, respectively. In context to the large volume preparation, the tonicity and osmoticity will be referred later in this thesis.

Protectants are also another type of excipients, which are added into a parenteral formulation to protect against loss of activity caused by some stress, from the manufacturing process. They can also prevent loss of active ingredient by packaging materials or by adsorption to process equipment.Attention should be given to packaging materials, rubber closures and other polymeric materials. They should be carefully examined for adsorptive potential; especially for intravenous infusions where most of the materials used (i.e. plastic bags, infusion sets, inline filters) are

polymeric. Human serum albumin (HAS) is used as protectant against adsorptive loss of proteins which may occur at lower concentrations. It prevents the adsorption of the drug by being preferentially adsorbed while coating the surface of interest and by being present at higher concentration (Florence and Siepmann2009).
Some examples of protectants are cryoprotectants and lyoprotectants and they are used to inhibit loss of the drug integrity resulting from freezing or drying, respectively. They also tend to improve the stability of proteins against inactivation by thermal denaturation.

All multiple-dose parenteral products should contain a suitable preservative system like **antimicrobial agents**, in bacteriostatic or fungi-static concentrations. They must be contained in adequate concentration when they are about to be used in order to prevent multiplication of microorganisms that were introduced into the preparation while withdrawing a portion of the contents with a hypodermic needle and syringe. However, the inclusion of preservatives can be a difficult challenge because of the viewpoints concerning which agents are acceptable to be used and when it is appropriate to include them in a formulation. Because of the fact that antimicrobial agents might be toxic for the patient, there are usually prescribed some maximum volume and concentration limits, particularly for those that are being frequently used in parenteral products.
Another task that the scientist will have to deal with while using antimicrobial agents is their interactions with other components of the formulation that could reduce the efficacy of the agents. It is also common that a particular agent will be effective in one formulation, but ineffective in another. For these reasons preservative efficacy testing should be done on each formulation they are used, to assure the effectiveness of the used concentration (Florence and Siepmann 2009; Troy 2005).
Despite all the challenges and difficulties that may occur while developing multi-dose formulations with antimicrobial preservatives, they offer several advantages such as: minimization of product wastage because different size doses may be obtained from the same container; also the same container may be used over a period

of time without the concern for microbial growth and last but not least the packaging is minimized because multiple doses are supplied in a single container.

Examples of the eight most commonly used antimicrobial preservatives are benzyl alcohol, chlorobutanol, m-cresol, methylparaben, phenol, phenoxyethanol, propylparaben, and thimerosal. Phenol and benzyl alcohol are more specifically used primarily in peptide and protein parenteral products. However, benzyl alcohol has been demonstrated to bind to and accelerate aggregation of proteins (associated with protein conformational denaturation or instability). Another example that was mentioned above, parabens, have a broad spectrum of antimicrobial activity at pH 4-8, but they are mainly used against yeasts and molds (Meyer et al, 2007).

5.1.2 Vehicles for parenterals

The most widely used vehicle for parenteral preparations, is the water for injection (WFI). WFI must be pure and pyrogen-free and that must be ensured while producing and storing it. WFI is usually obtained by distillation of deionized water and it is the only method that is permitted by the European Pharmacopoeia (EP), while the United States Pharmacopoeia (USP) and the Japanese Pharmacopoeia (JP) also permit reverse osmosis for its production.

Usual contaminants that are found in the water are microorganisms, dissolved organic and inorganic compounds, and foreign substances. Organic substances can be removed by charcoal beds while particulate contaminants can be removed by membrane and depth filters and inorganic compounds are usually removed deionization, reverse osmosis, distillation or a combination of the above methods. Last but not least microorganisms are reduced by filtration, recirculation of water, chilling or heating preventing by this way pyrogen to be formed that would have occurred in a static deionization system (Florence and Siepmann 2009).

If the WFI is going to be stored for more than 24 hours then it must be stored at temperatures like 5°Cor from 60°C to 90°C, in order to inhibit microbial growth. The USP also lists bacteriostatic WFI and sterile WFI. These must be sterile unlike WFI. Because of the possibility of glass leaching into the product during subsequent

storage and high-temperature sterilization, higher levels of solids are allowed in these vehicles. In order to prevent the administration of large quantities of bacteriostatic agents, bacteriostatic WFI should not be stored in containers larger than 30 ml (because if bacteriostatic agents are administered in large volumes, they can become toxic).

Solvents that are being used, other than the **aqueous** (i.e. water, ethanol in small volume parenterals), are the **non-aqueous** (i.e. soybean oil, safflower oil) and the **mixed** (aqueous/non-aqueous) ones. The last two may be necessary to stabilize drugs that are hydrolyzed by water or to improve their solubility. Non-aqueous solvents must be carefully screened and tested to make sure that they are non-toxic, non-irritating, they exhibit no pharmacological action and that they are stable and compatible with all the ingredients of a formulation (Florence and Siepmann 2009).

5.1.3 Large-Volume parenterals (LVPs)

Large-volume parenterals are pyrogen-free, sterile, aqueous solutions or emulsions with water as the continuous phase. They are usually made isotonic with respect to the blood and they are intended to be administered by intravenous infusion to replenish body fluids or electrolytes or to provide nutrition. They are administered in large volumes (100ml to 1000ml or more) per day by slow intravenous infusion and they are packed in large single-dose containers. Because of the large volumes they are being administered, they must not contain any antimicrobial preservatives or other pharmaceutical additives and when examined under suitable conditions of visibility they should be clear and free from particles. Electrolytes, vitamins and antineoplastics are frequently incorporated into large-volume parenterals for co-administration to the patient (Allen et al, 2005; Eur. Pharm. 8.0).

5.1.4 Types of LVPs

Large volume parenterals are designed in such way in order to provide electrolytes, fluid (water), carbohydrates (dextrose solutions) and some other uses that are going to be described below (Florence and Siepmann 2009).

Hyper-alimentation solutions are administered to patients intravenously, to provide them with large amounts of nutrients (i.e. carbohydrates, lipids, proteins and vitamins) in order to maintain them (also known as **maintenance therapy**) because they are unable to take food orally for several weeks (i.e. for amounts of 4000kcal/day or more).

Dextrose is the most commonly used carbohydrate, especially in high concentrations such as 20%; small amounts of fructose, alcohol and sorbitol can also be present in some products (dextrose solutions are all hypertonic and together with amino acid mixtures they exert the greatest effect on the osmolarity of the compounded product). In some special cases even insulin is provided. Providing large amounts of dextrose increases the solution's caloric value while at the same time keeps the volume that is required to be administered, to the minimum. Such solutions are administered slowly through a large vein i.e. the superior vena cava. This way, not only the rapid dilution of the concentrated hyper-alimentation fluid is achieved, as well as the risk of tissue or cellular damage due to hyper-tonicity of the solution, is minimized. In general, solutions that contain no more than 10% of dextrose can be administered peripherally, while those that contain more than 10% dextrose should be administered via the superior vena cava.

Sodium, potassium, magnesium, calcium, chloride, phosphate, and bicarbonate are some examples of the essential electrolytes that are used in relatively large amounts (Florence and Siepmann 2009; Allen et al, 2005).

Some examples of peptides and proteins that are being used in such drug formulations are the following: antivenin, desmopressin acetate, interferon alfa-n3 (derived from human leukocytes), insulin aspart (recombinant), insulin glargine (injection of rDNA origin), and epoetinalfa. Calcitonin-salmon injection (synthetic), etanercept, and sargramostim (recombinant) are used as well (Meyer et al, 2007).

Oil-in-water emulsions are heterogenous systems in which one liquid (internal phase) is dispersed throughout another (external or continuous phase) and they can offer a delayed absorption of the total dose, for drugs with large partition coefficient. Emulsions can solve problems such as insufficient drug solubility in water, or

hydrolysis of drugs. Similarly to all parenteral preparations, emulsions must meet many requirements regarding physic-chemical and biological stability, freedom from endotoxins, sterilization etc. A unique characteristic of parenteral emulsions is that they have strict requirements for globule size, as this has a direct effect on their stability and toxicity and it is directly affected by the oil concentration. The most frequently used globule sizes, are less than 1.0 – 2.0 μm, while sizes in the range 0.5 – 1.0 μm are utilized more rapidly by the body. Globule sizes higher than 4.0 – 6.0 μm are known to have an increased risk of causing emboli and changes in blood pressure. Once the emulsion is formed, it is necessary to reduce the globule size by using high-pressure homogenizers or microfluidizers. The oils that are being used for such preparations are either long-chain triglycerides (LCTs) obtained from vegetable sources (i.e. soybean oil, safflower oil, sesame oil, corn oil, castor oil etc.) or medium-chain triglycerides (MCTs) derived from re-esterification of fractioned coconut oil fatty acids. Emulsifiers that are being used are of either natural (derived from egg yolk or soybean oil) or synthetic (i.e. nonionic materials such as Pluronics – poloxamers or polyoxyethylene/polyoxypropylene derivatives-) origin. Despite the fact that natural emulsifiers may cause some allergic reactions, they are most frequently used because of their relative safety and stability. The synthetic ones are less frequently used, and mainly for small-volume parenterals because when used in large-volume parenterals or for long administration they can lead to "overloading syndrome" (Floyd, 1999).

It is important to mention that for the formulation of emulsions, the water soluble components are dissolved or dispersed in the aqueous phase first. Then the emulsifier can either be dissolved in the oil phase or dispersed in the aqueous phase. Antioxidants and lipophilic drugs are usually dissolved or dispersed in the oil phase (Floyd, 1999).

This is generally known as **total parenteral nutrition** (TPN) and should allow for anabolism, tissue synthesis and generally to maintain homeostasis in the body. This kind of formulations usually consists of dextrose mixtures, amino acids and lipids (i.e. soybean oil, safflower oil etc.), containing added trace metals, vitamins and

electrolytes. This method is applied to comatose patients or patients that undergo treatment for gastrointestinal tract diseases (including cancer), esophageal obstruction, ulcerative colitis, anorexia nervosa etc. and it is life-saving or life sustaining. Parenteral nutrition is also used from times to times in order to prepare a malnourished patient for surgery, chemotherapy or radiation therapy; and it is also used in patients with renal or hepatic failure. Long-term parenteral nutrition is also used in patients that have insufficient gastro-intestinal tissue for adequate digestion and absorption of nutrients, resulting from a surgery or a disease.It is used in patient's home (home parenteral nutrition, HPN) (Florence and Siepmann 2009).

Cardioplegia solutions are another type of LVPs used in heart surgery to prevent ischemic injury to the myocardium when the blood supply to the heart is clamped off. They are also used during reperfusion and to maintain an operating field free from blood as well as to make the myocardium flaccid. These are usually formulations that contain electrolytes, the composition of whose is intended to maintain diastolic arrest. Cardioplegia solutions are usually administered cold in order to minimize the metabolic activity and to cool the myocardium, after being admixed. They are usually hypertonic to minimize reperfusion injury (usually resulting from some tissue edema) and slightly alkaline to compensate for metabolic acidosis (Florence and Siepmann 2009).

The sterile **peritoneal dialysis solutions** are infused into the abdominal cavity, continuously, to clean the peritoneum and afterwards they are being continuously withdrawn. The peritoneum acts as a dialysis membrane for the exchange between the blood and dialysis solution. Those solutions aim to remove toxic substances from the body or to accelerate or aid the normal function of kidneys to excreting such substances. They also correct electrolyte imbalance, and eliminate fluid overload. This way they treat acute renal insufficiency as well as cases of drug or chemical toxicity. Their main content is dextrose and they are ionically similar to extracellular fluid. Toxins and metabolites are being removed by diffusing into the dialysis fluid

through the peritoneum. Antibiotics are commonly added into these solutions as a prophylactic measure (Florence and Siepmann 2009).

Last but not least peritoneal dialysis solutions do not restrict the patients on their diet or their fluid intake. Once the patients' electrolytes and fluids are corrected, they remain fairly constant and there is no significant anemia or blood loss. Only some protein might be lost through the peritoneum during dialysis, but this loss might be increased in case of peritonitis. Peritoneal dialysis solutions must be sterile and pyrogen-free. Since the peritoneal infection can be a recurrent problem, the patients must use strict aseptic techniques when exchanging the bags.

For irrigating, flushing and cleansing body cavities and wounds, **irrigating solutions** are being used. Because of the fact that these solutions are applied topically, they are not considered to be parenteral preparations. They are still considered large volume preparations though, and it is important to be mentioned. They must be sterile and pyrogen-free. These solutions are also used for surgical purposes (urological irrigating solutions). They remove blood and maintain tissue integrity during an operation. The water that is used for irrigation solutions is sterilized distilled water free from pyrogen and it is meant for a single use only. The container where it is packed is sealed and sterilized by moist heat.

Blood products are also commonly packed as sterile large volume parenteral fluids despite the fact that they are not considered to be large volume parenteral preparations. These products include albumin, human plasma and blood protein fractions. All these products must be treated with specialized heat or filtration, in order to inactivate virus contamination prior to packaging. Since these products are unstable to heat sterilization, they are filter-sterilized and then aseptically filled into containers (Florence and Siepmann 2009; Chapman 2009; Lund1994).

5.1.5 Uses of LVPs

Large volume parenterals are used in patients that are recovering from a surgery (or entering one) because they are unconscious or unable to take fluids, nutrition or electrolytes orally. They are also used in patients who have suffered a great loss of

electrolytes and fluid. These two types are also known as maintenance therapy and replacement therapy, respectively.

Maintenance therapy is usually employed when the patients are unable to receive nutrition or fluids orally for longer periods; this **total parenteral nutrition** is described in details earlier in this text (chapter 5.1.5).
It is recommended when infusing central or peripheral TPN solutions to use a filter in order to avoid precipitations that can cause serious hazards to the patient's health, such as pulmonary emboli caused by calcium phosphate precipitation. An example of such filter is a 0.22 μm filter that contains both bacterial-retentive and air-eliminating filters, and it is used in lipid-free (two-in-one) parenteral nutrients solutions. Two-in-one parenterals are used as an alternative to three-in-one admixtures, when using lipid emulsions (because they obscure any precipitate). In such case, lipids are infused separately into the two-in-one admixtures via a Y-site.

Replacement therapy is employed when the patient has suffered from severe diarrhea or vomiting greater than usual, and has lost great amounts of fluids and electrolytes. Patients with burns, trauma, Crohn's disease or AIDS are candidates for this type of therapy.

The daily **water requirement**, in normal adults, is the amount that is needed to replace normal and expected losses (i.e. losses from urine, feces, skin and respiration). Normal 'daily water requirement' values for adults are about 25 to 40 ml/kg of body weight. In water replacement therapy for adults, 70 ml/kg/day might be required in addition to water maintenance requirements. However, in order to avoid fluid overload, especially in patients with cardiovascular or renal disorders and in elderly, monitoring of their blood pressure is recommended. Administering water intravenously in such way may lead to osmotic hemolysis of red blood cells. To avoid this phenomenon and protect the red blood cells from hemolysis, water is generally administered as a solution with dextrose or electrolytes with sufficient

tonicity (because patients that require water, generally require nutrition and/or electrolytes).

For patients with **electrolyte requirement**, sodium, the primary extracellular cation and potassium, the principal intracellular anion**,** are most frequently administered.

Sodium is very important for maintaining extracellular fluids. It is conserved from the body when it is lost or removed from the diet. Negative sodium balance, when sodium levels are low, can be prevented by administering 3 to 5 g of sodium chloride to the patient. Low sodium levels in the body can be a result of excessive sweating, diarrhea or use of some diuretics. That can lead to muscle weakness, fatigue, apprehension and convulsions.

On the other hand, sodium levels can increase when the person suffers from some kidney impairment or if the person does not drink enough water (especially in hot weather). Symptoms of elevated sodium levels can be dry and sticky mucous membranes,flushed skin, thirst, lack of tears and elevated body temperature. High blood pressure is caused in 20% of the cases from sodium excess as well.

Sodium is usually paired with **chloride**, the primary extracellular anion. The importance of chloride is also significant, as it maintains the acid-base balance of the extra-cellular fluid, the fluid levels balance inside and outside the cell, and it also plays an important role in muscle contraction. Last but not least chloride is necessary to prevent bicarbonate from “moving” the acid-base balance towards the alkaline side.

Potassium is very important for maintaining normal cardiac and skeletal function. The usual daily potassium intake is about 100 mEq and the usual daily loss is about 40 mEq, so any replacement therapy should include at least 40 mEq plus the amount that is needed to replace the additional losses.

Potassium can be lost through several ways such as repeated enemas, excessive perspiration, different kinds of traumas, some gastrointestinal tract impairment, uncontrolled diabetes, surgery, and the use of some medications like loop diuretics

and thiazide. Some other reasons for low potassium levels might be anorexia nervosa, very low-calorie diets, or acute alcoholism because the patients are not taking in enough of it. General weakness, weak pulse, falling blood pressure and faint heart sounds are just some of the symptoms of low potassium levels in the organism. Severe potassium loss can even lead to death.

On the other hand, abnormally high potassium levels may cause diarrhea, pain, muscle cramps and irritability. Elevated potassium levels can be a result of excessive potassium-rich food consumption, or kidney failure. Other reasons for hyperkalemia may be potassium supplements, angiotensin-converting enzyme inhibitors, as well as the potassium-sparing diuretic therapy.

In severe potassium deficiency situations, intravenous electrolyte replacement with potassium chloride is usually employed. The amount as well as the infusion rate of the potassium chloride in the solution must be carefully checked; it must be carefully diluted with a large-volume parenteral solution, mixed well and administered by slow intravenous infusion. They must not be given undiluted, because this might lead to death. Last but not least, for patients in need of aggressive potassium replacement, the potassium serum level should be assessed every 6 hours during the early phase of therapy and later once normal potassium serum levels are achieved, it can be administered once per day.

In order to fulfill the **caloric requirements**, estimated by body weight, of patients that are undergoing maintenance or replacement therapy, a 5% dextrose is given to them. The use of dextrose is also minimizing ketosis as well as the protein breakdown.

Sometimes, although it is not recommended, it is necessary to administer **medication in combination** with the **parenteral nutrition** to ensure that the patient is receiving adequate nutrition as well as appropriate drug therapy. This mainly applies for young patients, who have a limited fluid capacity usually caused by some disease (i.e. renal insufficiency or congestive heart failure) and limited vascular access. It is also true

that administering the medication in combination with the parenteral nutrition rather than interrupting the feeding in order to administer the medication, makes the rebound hypoglycemia less likely. However, this practice has also some risks such as catheter sepsis and occlusion (Allen et al, 2005).

5.1.6 Sterilization methods

Sterile dosage forms have some standard characteristics that distinguish them from other dosage forms like the non-sterile. They must be safe, sterile, free from pyrogen contaminants, free from visible particulate matter, stable, compatible and isotonic (Florence and Siepmann 2009).

All those seven characteristics will be described in more detail in the following chapter (5.1.8). For sterilization five methods are usually used: steam, dry heat, filtration, gas, and ionizing radiation. They are mainly used for parenterals, although gas and ionizing radiation are mainly used for devices and surgical materials.Therefore, gas and ionizing radiation are not going to be mentioned in this chapter, since this text is mainly focused on the parenteral solutions, and not on the devices used for them.

Autoclave steam sterilization is a method that is being preferred when the solutions and the containers are able to withstand the autoclaving conditions, and it is used mainly for aqueous solutions. The main reasons are that moist heat sterilizes quickly as well as inexpensively. It is important that the solution and the container are permeable to steam though (i.e. oils and tightly closed containers are normally hard to sterilize by steam).

The use of steam sterilization has increased with the widespread use of flexible packaging for LVPs. In contrast to the traditional LVP glass bottles that are tightly closed with rubber stoppers, the flexible plastic LVP containers offer some advantages, regarding the autoclave sterilization. More specifically, they offer a larger surface area for heating per unit volume of liquid. Also, shorter heat-up and cool-down periods are required. Due to the net effect, LVP products that are packed

in flexible containers are allowed to have a shorter sterilization cycle, and by a shorter sterilization cycle, LVPs are exposed to less heat, having this way less potential to degradation. The manufacturing costs are also reduced. At last, if they are held in a "flattened" position during sterilization, then the heat penetration depth that is required is reduced, having as a result a more uniform thermal mapping of contents.

Another way of sterilizing, is **sterilization by dry heat**. This method has a good penetration power and is not as corrosive as steam. It is primarily used in powders and oily preparations. In contrast to steam sterilization, this method has a slow heat-up time, making long sterilization periods at high temperatures unavoidable.

The two principal methods of dry-heat sterilization are convection hot air and infrared. Convection hot-air sterilizers are usually heated electrically and are of two types: mechanical or gravity. In gravity convection units there is a need to promote uniformity of heat distribution throughout the chamber. In order to achieve that, a fan is used (Florence and Siepmann 2009).

Infrared rays on the other hand only sterilize surfaces. This method kills microorganisms mainly through oxidation. The amount of moisture that will be available to assist the sterilization in the dry-heat units varies in different locations of the chamber and at different time intervals within the cycle. Also the heat that will be available, its diffusion, and the environment at the air/spore interface can influence at some extent the kill rate of the microorganism. For this reason cycles tend to be longer and hotter to make sure that the varying conditions mentioned above will not invalidate a run.

Sterilization by filtration has become sufficiently reliable only in the past 30 years in order to be used on a wide scale to sterilize injectable solutions. The filters that are being used are of two basic types: depth and membrane.

Depth filters aim to retain particles and microorganisms through a combination of tortuous pathway and adsorption. Materials that are used to make such filters are

inorganic fibers, diatomaceous earth, natural fibers, and porcelain. Their biggest advantage is that they can retain very large quantitiesof particles. However, they also have disadvantages such as grow-through and reproduction of microorganisms, tendency of the filter components to slough during line surges, and also retention of some liquid in the filter.

Membrane filters on the other hand rely on sieving and absorption to prevent particles from passing, and their use has been widespread the last few years. These filters are also made from a variety of materials, although the most common ones are made of cellulose ester derivatives. Major advantages of membrane filters are no product retention, no media migration and efficiency that it is not dependent on low-rate pressure differential. Their disadvantages are low capacity before clogging and the need to remove surfactants by prewashing.

According to the advantages and disadvantages of both types, it is common to use a depth filter to remove the great majority of particles and afterwards to use a membrane filter in order to remove the remaining particles and microorganisms (Florence and Siepmann 2009).

5.1.7 Characteristics of sterile dosage forms

Sterile dosage forms have seven primary characteristics that make them unique pharmaceutical preparations. Those primary characteristics are going to be described below, in this chapter (Akers, 2005; Ph. Eur.8.0 2013).

Safety is the first to be mentioned as sterile dosage forms are mainly injected directly into the body, avoiding this way the body's barriers for anything that could be harmful to the body. For this reason, any compound to be injected into the body must be approved for its safety at the quantity that it is going to be injected (since any compound injected in large amounts can be unsafe). The formulation of sterile dosage forms is more difficult than the formulation of non-sterile dosage forms. That is because when it is needed to overcome a problem related to drug stability, solubility, tonicity etc. the requirement for safety prohibits the use of many effective

substances while for non-sterile dosage forms it is not necessary to restrict the options regarding to safety. Most pharmaceutical preparations are required to be tested for safety in animals, especially biological products, in order to provide additional assurance that they will not have any unexpected toxic properties (Akers, 2005).

Sterility is obviously what defines a sterile product. It is one of the greatest challenges to achieve and maintain sterility for such dosage forms. Sterility is achieved via various valid sterilization procedures for all components while manufacturing of the product, aseptic filtration, sterile environment where the products are being manufactured, use of antimicrobial preservatives for multiple-dose products, validation of aseptic processes and valid testing for sterility of the product as well as the maintenance of the container/closure integrity (Ph. Eur.8.0 2013).

Manufacturing a **pyrogen-free** product is very important since pyrogens are responsible for causing fever, and they originate mainly from microorganisms. In sufficient amounts they can also cause various complications to the human body and that is why all injectable products in the market must meet the requirements for pyrogen limits. Depyrogenation methods are rather complicating and they include cleaning validation, validated depyrogenation methods for glassware, time limitations, use of endotoxin-free materials, validated water systems, and removal of endotoxins from rubber closures (Akers, 2005).

Visible particulate matter has an impact on product quality and perhaps safety as well. Both ready-to-use and reconstituted solutions must be free from visible particulate matter and must meet the specifications for numbers of sub-visible particles no greater than specific sizes, usually no greater or equal to 10 μm or no

greater or equal to 25 μm (Akers, 2005; Ph. Eur.8.0 2013). Similarly to other sterility characteristics, the presence or absence of visible particulate matter depends on several factors. Such factors include valid cleaning methods and solution filtration procedures, adequate training of personnel in manufacturing and control of production, and testing of the products for both visible and sub-visible particulate matter.

Stability requirements apply for all dosage forms. All dosage forms must be stable under predetermined conditions during manufacturing, packaging, storage and usage. Both chemical and physical stability must be maintained throughout the shelf life of the product. Achieving and maintaining chemical and physical stability is the greatest challenge for the scientists developing sterile dosage forms; mainly the therapeutic peptides and proteins, because they are much more complicated chemical structures and they are more vulnerable to environmental conditions (i.e. pH, temperature, light, metal impurities etc.) (Akers, 2005).

Sterile products must be **compatible** with diluents for reconstitution and diluents for infusion. And since many infusions contain more than one drug (i.e. small volume parenterals) that means that all the drugs in the infusion must be compatible with each other. Despite the fact that most of the drugs do not require any manipulation prior to administration, there are still some sterile dosage forms that need to be manipulated either by the patient or by the health care professional. Such example are the freeze-dried products that need to be reconstituted by sterile dilution, withdrawn into a syringe and later they are usually combined with a large volume parenteral fluid in order to be ready for administration.

In ideal conditions any injected formulation should be **isotonic** with the human cells in order to avoid problems such as cell bursting (if the solution is hypotonic) or cell shrinking (if the solution is hypertonic). Large volume intravenous preparations and small volume preparations (administered in a way other than intravenously), must be

isotonic in order to avoid tissue irritation, pain and many other physiological reactions. Small volume injections do not have to be isotonic (although it would have been preferable) because small volumes do not damage a great amount of red cells that cannot be replaced. The ability of some solutes to dissociate into more than one species can explain the difference in isotonic concentrations among various large-volume solutions. For nonelectrolyte solutions that exist as a single entity (i.e. dextrose), it is true that their osmotic pressure is proportional to the concentration of the solute. On the other hand for electrolyte solutions that can be dissociated into two ionic species (i.e. sodium chloride), it is true that their osmotic pressure would be at least twice that of a solution containing a nonelectrolyte (Akers, 2005).

5.2 Osmosis & Osmotic properties

Osmotic effects play an important role in maintaining the homeostasis (equilibrium in body regarding the chemical composition of fluids and tissues as well as various body functions i.e. blood pressure, heart rate etc.). It is not easy to measure these effects because they occur within or between cells and tissues (Allen, 2013). Osmosis can be expressed as a movement of solvent molecules through a partially permeable membrane (permeable to the solvent, but not the solute) from regions of lower solute concentration to regions of higher solute concentration, resulting to the equilibrium of the solute concentrations on the two sides. The pressure that is required to achieve and maintain this equilibrium, without any solvent movement, is defined as the *osmotic pressure* and is a very important parameter in all biological processes that involve fluid transfer, through biological membranes. An example of the importance of the osmotic pressure, is that its knowledge allows the practitioners to determine whether a parenteral solution is hyperosmotic, iso-osmotic or hypo-osmotic (USP 35/NF 30 2011).

5.2.1 Osmotic Pressure

Each particle in a solution (regardless of its mass) exerts on average the same amount of pressure against the membrane. Thus, the osmotic pressure exerted by non-diffusible particle of a solute in a solution, is determined by the number of particles

per unit volume of the fluid and not by the mass of the material. The osmotic pressure is a colligative property. This means that it depends on the number of particles and (molal concentration) in the solution. Particles can be un-ionized molecules, ions, macromolecules, or aggregates (i.e. a dimer). For ideal solutions where the solutes are non-dissociating (solutes and solvent do not interact with each other), the osmotic pressure πcan be estimated directly from the molality according to the following equation (USP 35/NF 30 2011):

$$\pi = (d \cdot R \cdot T / 1000) \cdot m \quad (1)$$

Where d is the density (kg/l), R is the ideal gas constant (8.314 $J / mol \cdot K$), T is the absolute temperature (^{o}K) and m is the molality (mol/kg). For a real solution (non-ideal behaviour), where the solute(s) is/are being dissociated the osmotic pressure can be calculated from the formula (USP 35/NF 30 2011):

$$\pi = (d \cdot R \cdot T / 1000) \cdot \Sigma \upsilon \cdot m \cdot \Phi_m \quad (2)$$

Where d, R, T are as mentioned above, υis the number of particles formed after dissociation of one molecule (i.e. for NaCl υ=2), and Φ_m is the molal osmotic coefficient. It depends on the ionic characteristics of the solute, itsconcentration and its chemical properties. It can be experimentallydetermined by measuring the freezing point depression at different molal concentrations. Its value is less than one for concentrations of pharmaceutical interest and it decreaseswhen the molal concentration of the solute increases. From the equation (2) it can be seen that the factor that determines the osmotic pressure of a solution is the concentration of the solution in terms of number of particles and not in terms of the mass of the solute (USP 35/NF 30 2011).

The osmotic pressure effects are very dynamic. For example the time that is required to achieve osmotic equilibrium between compartments in tissues, is usually just a few seconds (maximum a minute or two). For the body as a whole, more time is needed though. For example for a normal person it would require thirty to sixty minutes to achieve reasonably good osmotic equilibrium after drinking water. It is important to be mentioned that body fluids like blood and lacrimal fluid, normally

have an osmotic pressure which is described as corresponding to that of 0.9% solution of sodium chloride (Deardorff, 1980).

5.2.2 Osmolality and Osmolarity

It is very important to consider the concentration of solutes in osmotic relationships, when preparing large-volume parenteral preparations, because they are expected not to cause any undesired side effects on the body tissue. Intravenous fluids, in order to be safely applied into the body, they should be formulated with an osmotic pressure similar to biological fluids. This is largely determined by the osmotic effect of the dissolved solutes, and for this reason osmolarity should be listed on the labelling.

The concentration of the osmotically active particles in a solution can be expressed either by osmolality m_{os}(the osmolal concentration in Osmol/kg or mOsmol/kg) or by osmolarity c_{os} (the osmolar concentration in Osmol/l or mOsmol/l).Osmolality, similar to the osmotic pressure, is directly connected to other colligative properties of a solution likevapour pressure lowering, boiling point elevation, and freezing point depression. It is true that the most accurate way of determining osmolality, is by measuring the freezing point depression:

$$m_{os} = \frac{\Delta T}{1.86} \cdot 1000 \qquad (3)$$

Where ΔT is the freezing point depression measured in absolute temperature and $m_{os}$the osmolality as mentioned above in the text.

In practice the measurement of osmolality is performed by an a special measuring device called osmometer, which consists of a means of cooling the container used for the measurement,a thermistor, a mixing device (vibrator) and a glass vessel where the sample to be measured is placed. Before the measurement the osmometer has to be calibrated.At the beginning,the cooling system is being started. And after a while, the mixing device (vibrator) is engaged at a temperature below the lowest temperature expected from the freezing point depression to prevent supercooling. The results of this measurement are given in mOsmol/kg by a digital readout (Knauer User's manual).

Osmolality is generally easy to determine in laboratory, however, osmolarity is the value that needs to be labelled in parenteral preparations. Osmolarity c_{os} cannot be determined experimentally but it can be calculated theoretically, from the laboratory values of osmolality and from a method called "water content". Some values that are required for such calculations are the density of the solutions, the volume of the solvent, and the partial specific volume of the solvent.

The first method to calculate the osmolarity is the theoretical method according to the following equation:

$$c_{os} = \Sigma \upsilon_i \cdot c_i \qquad (4)$$

Where υ is the number of dissociating particles (i.e. υ=2 for sodium chloride, υ=2 for potassium chloride etc.), and c is the molarity in mol/l. However, large errors can occur from this equation even for relatively simple electrolyte systems. The more the concentration increases or more complex the system is, the more the possibility of error increases (20% or even more). This happens because the above equation (4) describes a solution with an ideal behavior where the particles do not dissociate and therefore do not interact with the solvent or with each other, which is not true for real solutionscontaining dissociating particles (Deardorff, 1980).

Another way of estimating the osmolarity c_{os}, is by using values such as the experimentally determined osmolality m_{os} (mOsmol/kg), the also experimentally determined density d (g/ml) and the partial specific volume V_g:

$$c_{os} = \frac{1000 \cdot m_{os}}{\frac{1000}{d} + \Sigma M_0 \cdot V_g} \qquad (5)$$

Where d is the density (g/ml), M_0 is the mass of the substance (g), and $V_g$is the partial specific volume (ml/g). The partial specific volume of a solute is defined as the volume change of a solution when an additional 1g of a solute is dissolved in the solution.

At last, one more method of measuring the osmolarity c_{os}, is the method also known as "water content" method:

$$c_{os} = m_{os} \cdot (d - C) \qquad (6)$$

Where d is the density (g/ml), and C is the total solute concentration (g/ml) (USP 35/NF 30 2011).

6 Experimental section

6.1 Materials

Sodium chloride (NaCl) (Ph. Eur. 6.2, Kulich Pharmas.r.o)

Potassium chloride (KCl) (Ph. Eur 5.0, Kulich Pharmas.r.o)

High quality distilled water for HPLC (Faculty of Pharmacy, Hradec Kralove)

6.2 Equipment

Moisture analyser, PRECISA 330 XM, Switzerland, d = 0.001 g

Analytical balance, KERN ABJ 120-4M (Kern &Sohn GmbH), Germany, d = 0.0001 g

Electronicalbalance, ACCULAB Atilon ATL-4202-V, Sartorius Group, Germany, d = 0.01 g

Density meter with oscillating U-tube installed (DMA 4100 M, Anton Paar, Austria)

Osmometer(automatic semi-micro, Knauer, Germany)

Eppendorf pipette (0-200µl, Eppendorf, Germany)

6.3 Methods

6.3.1 Preparation of solutions

For the experiment I had to prepare solutions of sodium chloride and potassium chloride in molality concentrations: 0.01, 0.02, 0.03, 0.04, 0.05, 0.06, 0.07, 0.08, 0.09, 0.10, 0.50 and 1.00 mol/kg each. To achieve those concentrations first I had to dry some amount of the substance to be used on moisture analyser, at 105^{o}C for 30 minutes.

Then I measured different mass values for each substance on the analytical balance with precision of 0.0001g. Each measured mass of substancewas dissolvedin exactly

weighed1.00 kg of high quality distilled water and placed afterwards into closed bottles to prevent any evaporation.

Later, I used the raw prepared samples for measurement of density and measurement of osmolality.

6.3.2 Measurement of density

For measurement of density the first step was to check the air/water density and temperature,at 20°C under the atmospheric conditions (15 minutes after the device was turned on). High quality distilled water was exposed to ultrasound in order to eliminate any possible gas that was "trapped" inside for 5 minutes.

Afterwards each sample was measuredat 20°C, by introducing 1 ml the solution into the oscillating U-tube of the density meter with a syringe. This procedure was repeated 5 times for each one of the solutions.

According to the instruction manual in the end of the measurements the density meter had to be rinsed 3 times with distilled water, and afterwards to pump air inside with a syringe 3 times as well. The last step was to engage the fan of the density meter which was "On" for approximately 15 minutes. This plays a significant role in the drying procedure (drying the tubes) of the density meter.

The results of this measurement will be found in Chapter 7. (Table 1 and Table 2).

6.3.3 Measurement of osmolality

For measurement of osmolality,the osmometer had to be calibrated in first place, according to Ph. Eur. 8.0 2013. To perform the calibration reference solutions of sodium chloride with ideal osmolalities 100, 200, 300, 400, 500, 600, and 700 mOsmol/kg had to be prepared. Then the real osmolalities were determined by using the equipment. Finally,the calibration equation was expressed to convert the measurement results of m_{os} (mOsmol/kg) to the ideal osmolalities (mOsmol/kg).

The 1stcalibration equation with coefficient of determination $R^2 = 0.9998$ is:

$$y = 0.9684\ x + 2.2 \qquad (7)$$

where y = the measured m_{os} and x = the ideal m_{os}

The 2nd calibration equation with coefficient of determination $R^2 = 0.9998$ is:

$$y = 1.002381\ x + 0.166667 \quad (8)$$

where y = the measured m_{os} and x = the ideal m_{os}

In order to check the equipment at every day measurement, first,the checking was done with water (0 mOsm/kg). 150µl of water were put in the clean, dry glass vessel with a pipette, was let to cool before the measurement started. After the water, one more check was performed with a standard solution (400 mOsm/kg). After those procedures were done, it was time to measure the osmolality of the samples.

150µl of the sample were introduced to the osmometervessel and waited for approximately 1 minute until the temperature equilibrium of the sample solution was achieved. Then, the measurement started by pushing the Start button. After the measurement was finished, some waiting was needed for the sample to defreeze, and afterwards it had to be mixed (with a special vibration device, inside the osmometer) and repeat the measurement (5 times for each sample).

When switching from one sample to another, it is required to wash with distilled water and then dry the measuringhead of the osmometer and the glass wessel where the sample was placed.

The results of this measurement will be found in Chapter 7. (Table 3 and Table 4).

6.4 Manipulation of results

6.4.1 Conversion molality to molarity

To convert molality m (mol/kg) to molarity c (mol/l), I divided the molality with the volume V (l) of the solution according to the following equation (Sklubalova and Zatloukal 2009):

$$c = \frac{m}{V} \quad (9)$$

The results of this measurement will be found in Chapter 7 in Table 5 and Table 6 for sodium chloride and potassium chloride, respectively.

6.4.2 Estimation of partial specific volume

The partial specific volume $V_g$of a solute, is defined as a volume change of a solution when an additional 1g of a solute is dissolved in it (USP 35/NF 30 2011;).

The partial specific volume can be calculated from the following equation:

$$V_g = \frac{V - V_w}{M_0} \tag{10}$$

Where V_g is the partial specific volume in ml/g, V is the volume of the solution in ml, V_w is the volume of water in ml and M_0 is the mass of the solute in g.

The results of this measurement will be found in Chapter 7 Table 5/Figure 5 and Table 6/Figure 7 for sodium chloride and potassium chloride, respectively.

6.4.3 Estimation of molal volume

Molal volume V_{mol} (ml/mol) is the volume of 1 mol of a solvent at specific temperature and pressure conditions. The molal volume can be calculated similarly to the $V_g$from the following equation:

$$V_{mol} = \frac{V - V_w}{m} \tag{11}$$

Where $V_{mol}$is the molal volume in ml/mol, V is the volume of the solution in ml, V_w is the volume of water in ml and m is the molality of the solvent in mol/kg.

The results of this measurement will be found in Chapter 7 Table 5/Figure 6 and Table 6/Figure 8 for sodium chloride and potassium chloride, respectively.

6.4.4 Estimation of molal osmotic coefficient

The molal osmotic coefficient expresses the deviation of a solution from ideal behavior. The molal osmotic coefficient can be calculated by the following equation (USP 35/NF 30 2011; Ph. Eur. 7.3):

$$\Phi_m = \frac{m_{os}}{\upsilon \cdot m} \tag{12}$$

Where $\Phi_m$is the molal osmotic coefficient, m_{os} is the osmolality (osm/kg), υ is the number of particles formed by dissociation of one molecule (in the case of sodium

chloride and potassium chloride, this value is equal to 2), and m is the molality in mol/kg.

The results of this measurement will be found in Chapter 7 Table 7/Figure 9 and Table 8/Figure 10 for sodium chloride and potassium chloride, respectively.

6.4.5 Conversion osmolality to osmolarity

The osmolarity c_{os}(osmol/l), can be calculated in three different ways (USP 35/NF 30 2011).

One of them is the ***theoretical osmolarity*** (see Eq. 4) and takes in consideration only the number of particles formed by dissociation of a molecule (in the case of sodium chloride and potassium chloride, this value is equal to 2), and the molar concentration c (mol/l).

Osmolarityc_{os} (osmol/l) can also be calculated by the experimentally determined osmolalitym_{os} (osmol/kg) and the ***partial specific volume*** of substance V_g(ml/g), by the equation (5) (unless the solution is very concentrated):

Last but not least, osmolarityc_{os} can be calculated by the so called "***water contentmethod***" according to the equation (6).

The results of osmolarity estimation will be found in Chapter 7 Table 7/Figure 11 and Table 8/Figure 12 for sodium chloride and potassium chloride, respectively.

7 Results

Table 1: Results of density measurements of sodium chloride solutions

m (mol/kg	Results of density					Average	SD
0.01	0.9986	0.9986	0.9986	0.9986	0.9986	0.9986	0.00
0.02	0.9990	0.9990	0.9990	0.9990	0.9990	0.9990	0.00
0.03	0.9995	0.9995	0.9995	0.9995	0.9995	0.9995	0.00
0.04	0.9999	0.9999	0.9999	0.9999	0.9999	0.9999	0.00
0.05	1.0003	1.0003	1.0003	1.0003	1.0003	1.0003	0.00
0.06	1.0007	1.0007	1.0007	1.0007	1.0007	1.0007	0.00
0.07	1.0011	1.0011	1.0011	1.0011	1.0011	1.0011	0.00
0.08	1.0015	1.0015	1.0015	1.0015	1.0015	1.0015	0.00
0.09	1.0019	1.0019	1.0019	1.0019	1.0019	1.0019	0.00
0.10	1.0023	1.0023	1.0023	1.0023	1.0023	1.0023	0.00
0.50	1.0185	1.0185	1.0185	1.0185	1.0185	1.0185	0.00
1.00	1.0378	1.0378	1.0378	1.0378	1.0378	1.0378	0.00

Table 2: Results of density measurements of potassium chloride solutions

m (mol/kg)	Results of density					Average	SD
0.01	0.9987	0.9987	0.9987	0.9987	0.9987	0.9987	0.00
0.02	0.9992	0.9992	0.9992	0.9992	0.9992	0.9992	0.00
0.03	0.9996	0.9996	0.9996	0.9996	0.9996	0.9996	0.00
0.04	1.0001	1.0001	1.0001	1.0001	1.0001	1.0001	0.00
0.05	1.0006	1.0006	1.0006	1.0006	1.0006	1.0006	0.00
0.06	1.0011	1.0011	1.0010	1.0010	1.0010	1.00104	0.00
0.07	1.0015	1.0015	1.0015	1.0015	1.0015	1.0015	0.00
0.08	1.0020	1.0020	1.0020	1.0020	1.0020	1.002	0.00
0.09	1.0025	1.0025	1.0025	1.0025	1.0025	1.0025	0.00
0.10	1.0029	1.0029	1.0029	1.0029	1.0029	1.0029	0.00
0.50	1.0211	1.0211	1.0211	1.0211	1.0211	1.0211	0.00
1.00	1.0428	1.0429	1.0429	1.0429	1.0429	1.04288	0.00

Figure 1: Graph showing the connection between density-molality for sodium chloride

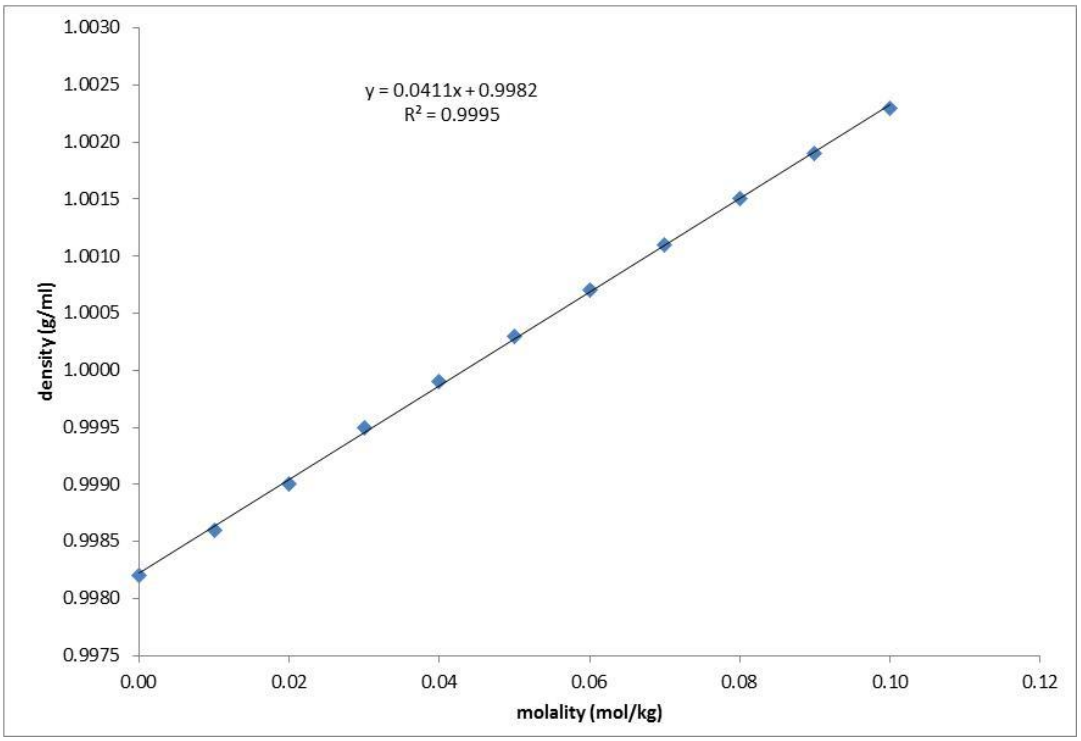


Figure 2: Graph showing the connection between density-molality for potassium chloride

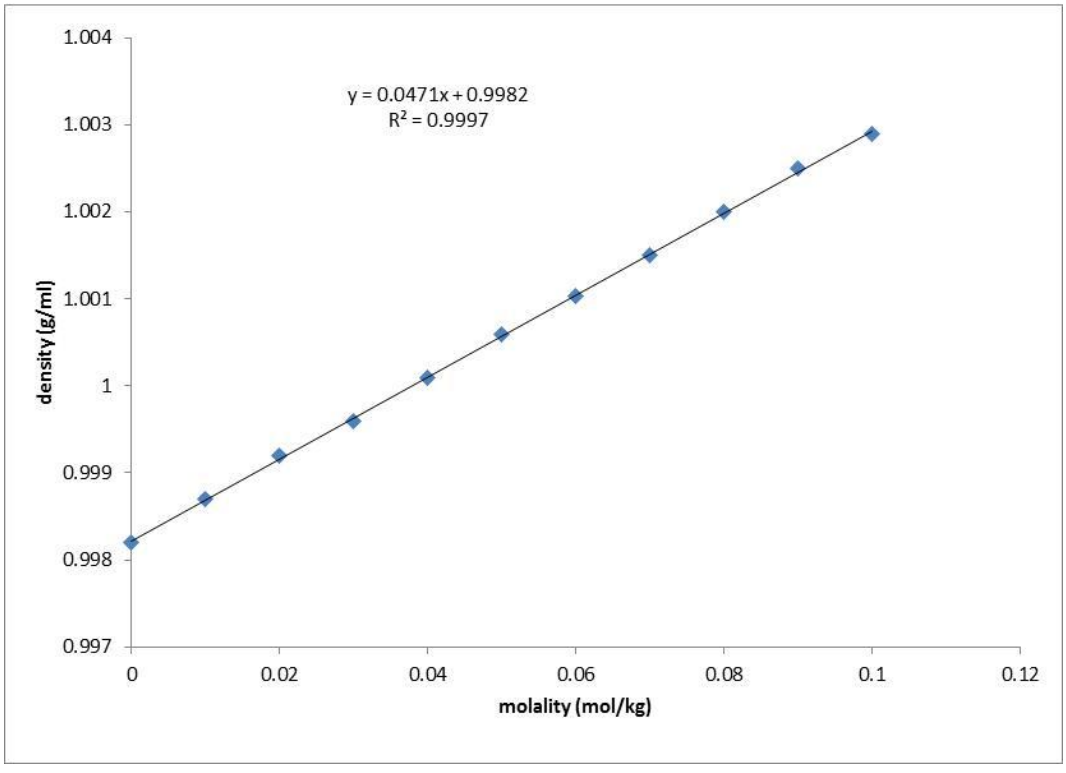


Table 3: Results of osmolality measurements of sodium chloride

m (mol/kg)	Results of osmolality					Average	SD	Ideal m_{os}
0.01	19	19	19	20	19	19.2	0.45	17.6
0.02	38	38	38	38	37	37.8	0.45	36.8
0.03	57	57	58	58	58	57.6	0.55	57.2
0.04	73	74	71	74	75	73.4	1.52	73.5
0.05	93	94	92	93	94	93.2	0.84	94.0
0.06	113	114	112	115	113	113.4	1.14	114.8
0.07	130	134	135	134	134	133.4	1.95	135.5
0.08	150	144	151	150	153	149.6	3.36	152.2
0.09	170	167	168	169	168	168.4	1.14	171.6
0.10	175	180	178	180	178	178.2	2.05	181.7
0.50	888	909	906	902	906	902.2	8.32	929.3
1.00	1763	1764	1772	1777	1780	1771.2	7.60	1826.7

Table 4: Results of osmolality measurements of potassium chloride

m (mol/kg)	Results of osmolality					Average	SD	Ideal m_{os}
0.01	20	20	20	19	20	19.8	0.45	19.6
0.02	38	40	39	39	40	39.2	0.84	38.9
0.03	57	58	57	57	56	57.0	0.71	56.7
0.04	77	77	77	75	77	76.6	0.89	76.3
0.05	93	94	95	95	96	94.6	1.14	94.2
0.06	115	115	115	115	115	115	0.00	114.6
0.07	131	129	128	131	131	130	1.41	129.5
0.08	152	151	152	152	150	151.4	0.89	150.9
0.09	170	171	167	167	170	169	1.87	168.4
0.10	187	188	186	187	188	187.2	0.84	186.6
0.50	884	882	881	882	880	881.8	1.48	879.5
1.00	1716	1722	1716	1722	1722	1719.6	3.29	1715.3

Figure 3: The relationship between osmolality-molality for sodium chloride

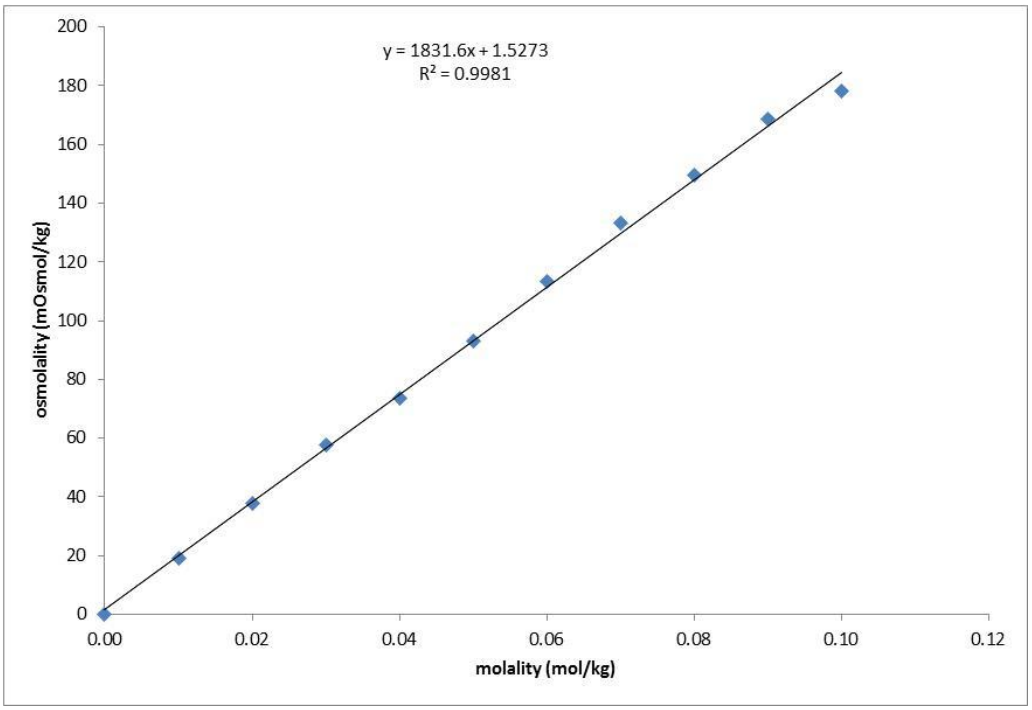


Figure 4: The relationship between osmolality-molality for potassium chloride

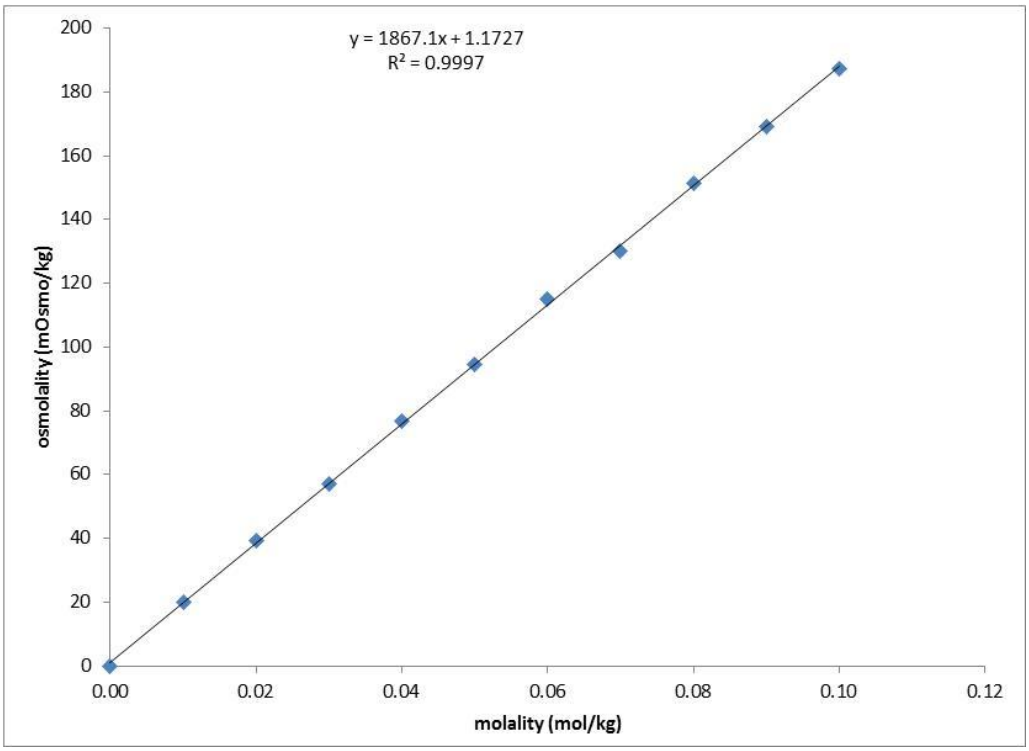


Table 5: The properties of sodium chloride solutions

m (mol/kg)	M_0 (g)	M (g)	d (g/ml)	V (ml)	c (mol/l)	V_g (ml/g)	V_{mol} (ml/mol)
0.01	0.5844	1000.5844	0.9986	1001.9872	0.0100	0.31	18.39
0.02	1.1688	1001.1688	0.9990	1002.1710	0.0200	0.31	18.39
0.03	1.7532	1001.7532	0.9995	1002.2543	0.0299	0.26	15.04
0.04	2.3376	1002.3376	0.9999	1002.4378	0.0399	0.27	15.86
0.05	2.9220	1002.9220	1.0003	1002.6212	0.0499	0.28	16.36
0.06	3.5064	1003.5064	1.0007	1002.8044	0.0598	0.29	16.69
0.07	4.0908	1004.0908	1.0011	1002.9875	0.0698	0.29	16.92
0.08	4.6752	1004.6752	1.0015	1003.1704	0.0797	0.29	17.09
0.09	5.2596	1005.2596	1.0019	1003.3532	0.0897	0.29	17.22
0.10	5.8440	1005.8440	1.0023	1003.5359	0.0996	0.30	17.33
0.50	29.2200	1029.2200	1.0185	1010.5253	0.4948	0.30	17.44
1.00	58.4400	1058.4400	1.0378	1019.8882	0.9805	0.31	18.08

Table 6: The properties of potassium chloride solutions

m (mol/kg)	M_0 (g)	M (g)	d (g/ml)	V (ml)	c (mol/l)	V_g (ml/g)	V_{mol} (ml/mol)
0.01	0.7455	1000.7455	0.9987	1002.0482	0.010	0.33	24.49
0.02	1.4910	1001.4910	0.9992	1002.2928	0.020	0.33	24.48
0.03	2.2365	1002.2365	0.9996	1002.6376	0.030	0.37	27.81
0.04	2.9820	1002.9820	1.0001	1002.8817	0.040	0.36	26.96
0.05	3.7275	1003.7275	1.0006	1003.1256	0.050	0.35	26.45
0.06	4.4730	1004.4730	1.00104	1003.4294	0.060	0.36	27.10
0.07	5.2185	1005.2185	1.0015	1003.7129	0.070	0.37	27.28
0.08	5.9640	1005.9640	1.002	1003.9561	0.080	0.36	26.91
0.09	6.7095	1006.7095	1.0025	1004.1990	0.090	0.36	26.62
0.10	7.4550	1007.4550	1.0029	1004.5418	0.100	0.37	27.39
0.50	37.2750	1037.2750	1.0211	1015.8408	0.492	0.38	28.08
1.00	74.5500	1074.5500	1.04288	1030.3678	0.971	0.38	28.56

Figure 5: Graph showing the connection between partial specific volume (V_g) and molality for sodium chloride

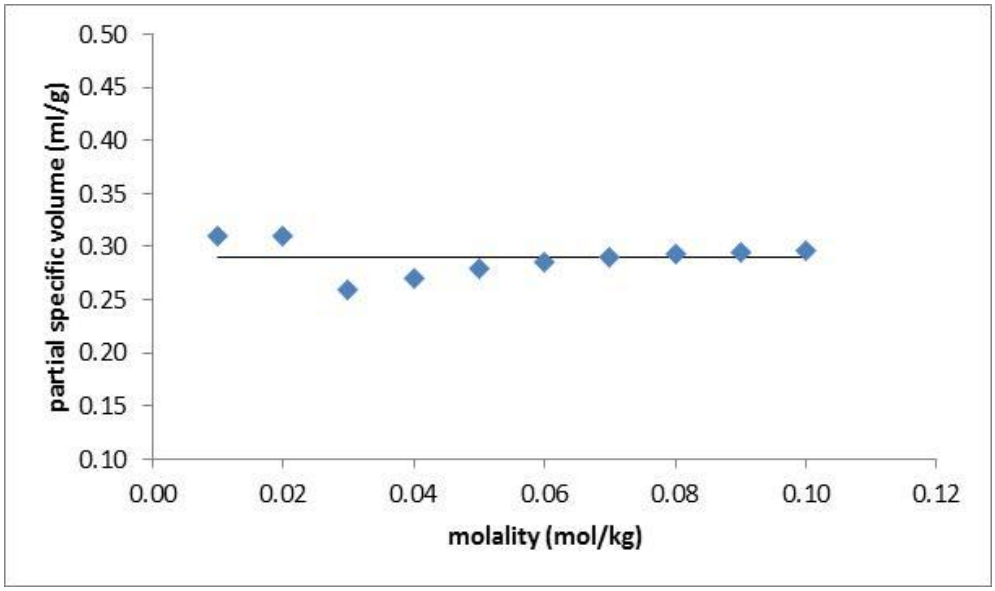


Figure 6: Graph showing the connection between molal volume (V_{mol}) and molality for sodium chloride

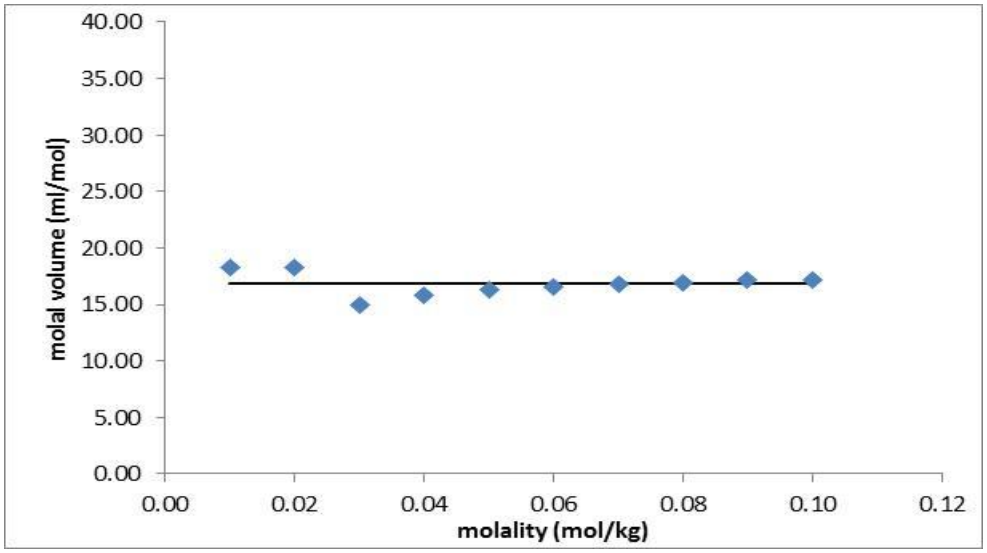


Figure 7: Graph showing the connection between partial specific volume (V_g) and molality for potassium chloride

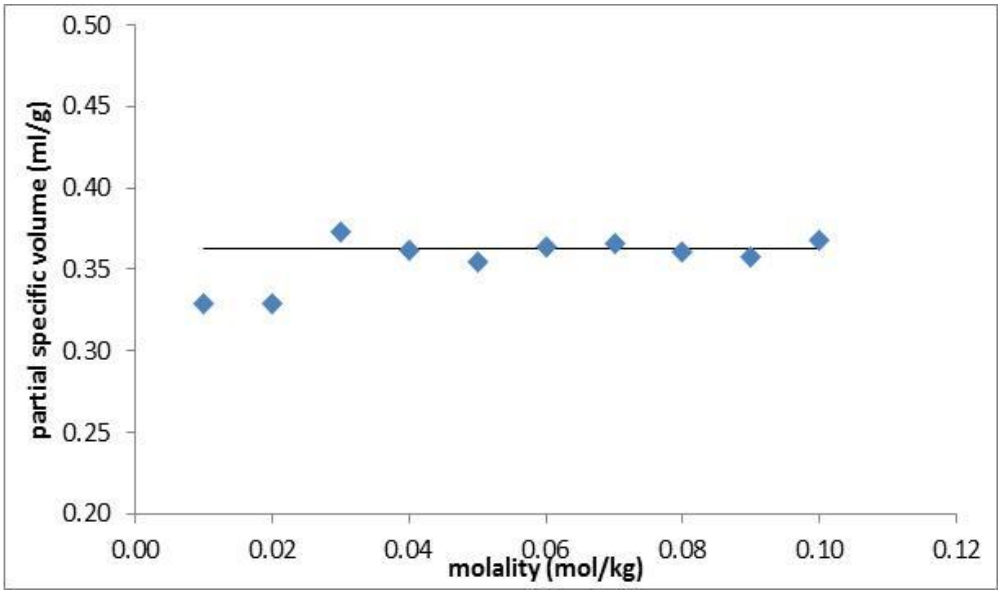


Figure 8: Graph showing the connection between molal volume (V_{mol}) and molality for potassium chloride

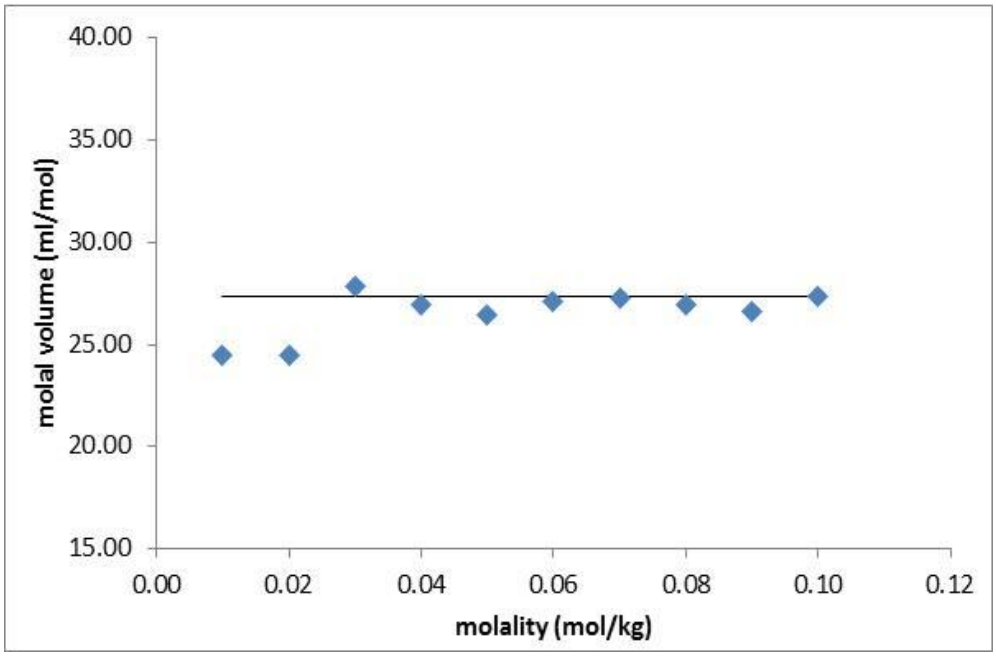


Table 7: Approximation of osmolarity of sodium chloride solutions

			c_{os} (mOsmol/l)		
m (mol/kg)	m_{os}(mOsmol/kg)	Φm	Theoretical (Eq.4)	Experimental (V_gEq.5)	(w.c.Eq.6)
0.01	19.2	0.960	20.0	17.5	17.5
0.02	37.8	0.945	39.9	36.7	36.7
0.03	57.6	0.960	59.9	57.1	57.1
0.04	73.4	0.918	79.8	73.4	73.3
0.05	93.2	0.932	99.7	93.9	93.7
0.06	113.4	0.945	119.7	114.8	114.5
0.07	133.4	0.953	139.6	135.5	135.1
0.08	149.6	0.935	159.5	152.2	151.7
0.09	168.4	0.936	179.4	171.7	171.0
0.10	178.2	0.891	199.3	181.8	181.1
0.50	902.2	0.902	989.6	938.2	919.4
1.00	1771.2	0.886	1961.0	1860.8	1789.2

Table 8: Approximation of osmolarity of potassium chloride solutions

			c_{os} (mOsmol/l)		
m (mol/kg)	m_{os}(mOsmol/kg)	Φm	Theoretical (Eq.4)	Experimental (V_gEq.5)	(w.t. Eq.6)
0.01	19.8	0.990	20.0	19.6	19.5
0.02	39.2	0.980	39.9	38.9	38.9
0.03	57	0.950	59.8	56.6	56.5
0.04	76.6	0.958	79.8	76.2	76.0
0.05	94.6	0.946	99.7	94.1	93.9
0.06	115	0.958	119.6	114.5	114.2
0.07	130	0.929	139.5	129.5	129.0
0.08	151.4	0.946	159.4	150.9	150.3
0.09	169	0.939	179.2	168.4	167.7
0.10	187.2	0.936	199.1	186.6	185.7
0.50	881.8	0.882	984.4	885.4	865.4
1.00	1719.6	0.860	1941.1	1737.2	1661.3

Figure 9: Graph showing the connection between molal osmotic coefficient Φ_m-molality for sodium chloride

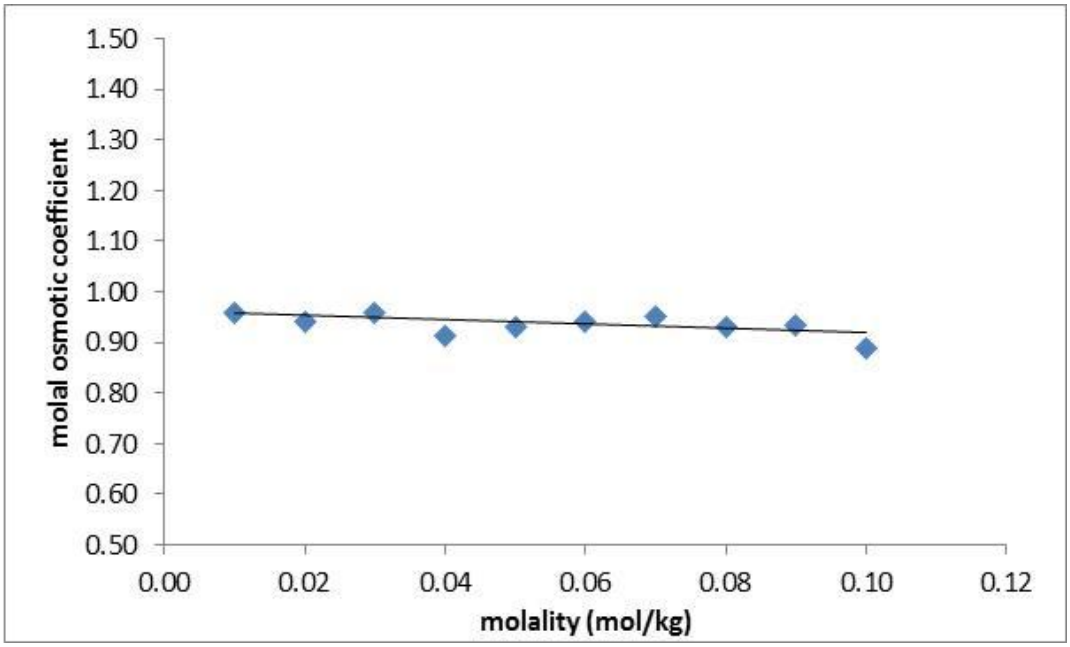


Figure 10: Graph showing the connection between molal osmotic coefficient Φ_m-molality for potassium chloride

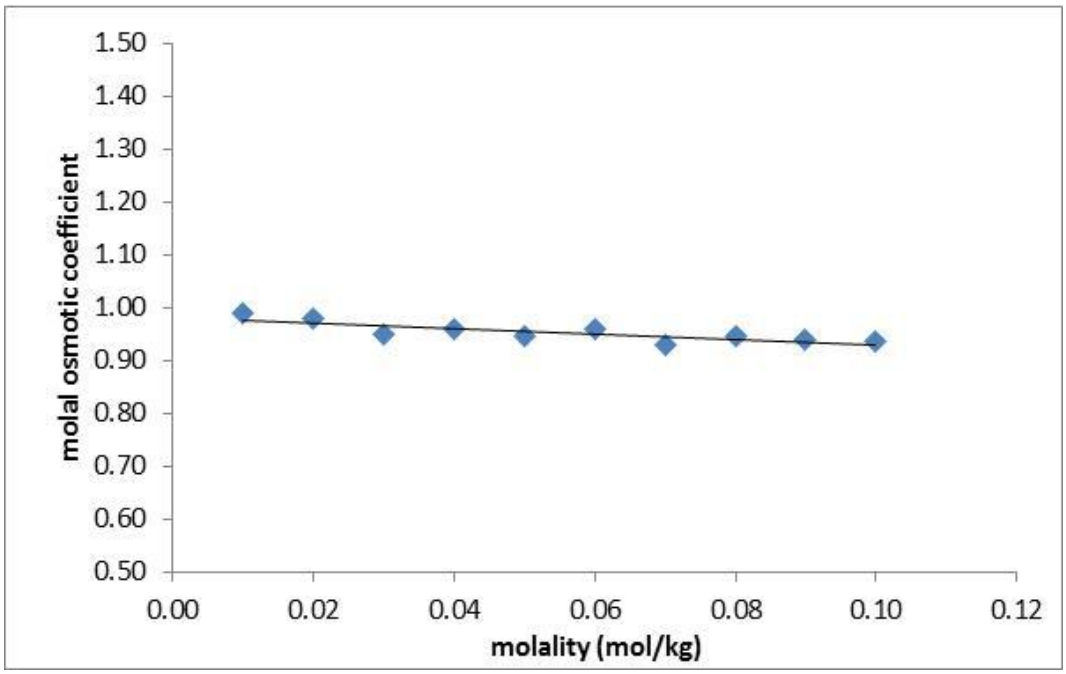


Figure 11: Relationship between osmotic concentrations and molality for sodium chloride (c_{os} (1) is calculated by Eq. 4, c_{os} (2) is calculated by Eq. 5, and c_{os} (3) is calculated by Eq. 6)

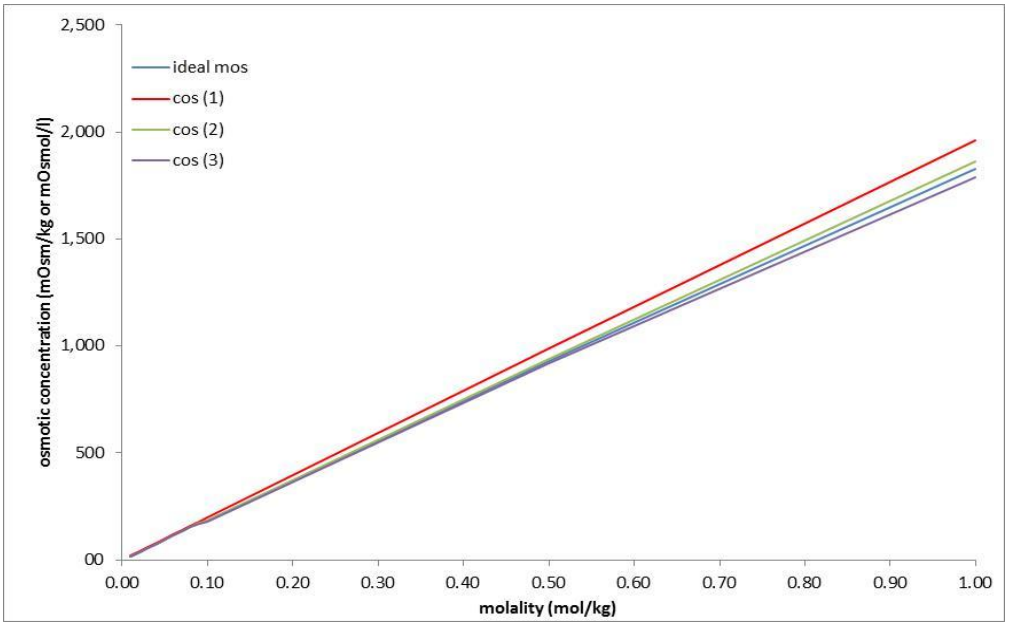
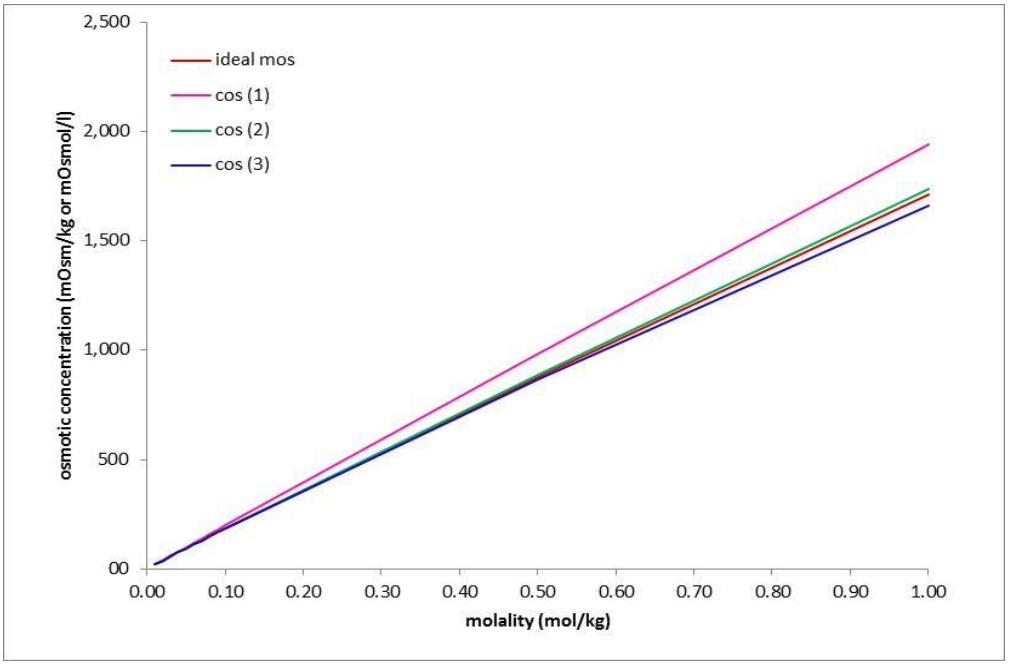


Figure 12: Relationship between osmotic concentrations and molality for potassium chloride(c_{os} (1) is calculated by Eq. 4, c_{os} (2) is calculated by Eq. 5, and c_{os} (3) is calculated by Eq. 6)



8 Discussion

The importance of the osmotic effects in the organism is very big because they are responsible for maintaining the human body homeostasis. More specific, osmotic pressure is very significant for all biological processes that involve fluid transfer through biological membranesand therefore maintaining the "tone" of the cells; this includes a specific biological concentration of ions, molecules, and aggregated species that give the cells specific properties.

In this experimental work aqueous solutions of sodium chloride and potassium chloride were prepared in different molality concentrations in a range of 0.01 – 1.00 mol/kg. Those solutions were used to measure their density and molality in a procedure that was described above in this text. The results that were obtained from those measurements were used to convert molality to molarity as well as osmolality to osmolarity.

The results are summarized in Tables 1 – 8 and Figures 1 – 12. In the figures, only the results in a concentration range of 0.01 – 0.10 mol/kg are presented, because it was difficult to plot them all in the same graph, due to the large difference in scale.

Density

The density was measured with a density meter the principle of which is measuring the frequency of oscillation. The results are summarized in Tables (1) and (2). The relationship between molality m (mol/kg) and density d (g/ml) follows a simple proportion that can be seen on Figures (1) and (2), respectively. The results were in accordance to the theory.

From those results it was also possible to generate the equations for both sodium chloride and potassium chloride that show this relationship. For sodium chloride the equation with coefficient of determination ($R^2 = 0.9995$) is:

$$d = 0.0411 \cdot m + 0.9982 \quad (13)$$

and for potassium chloride the equation with coefficient of determination ($R^2 = 0.9997$)is:

$$d = 0.0471 \cdot m + 0.9982 \quad (14)$$

Using the equations, it is possible to calculate any density value (y axis) for any molality value (x axis) within the range from 0.00 mol/kg to 1.00 mol/kg.

Osmolality

Osmolality was measured with an osmometer by measuring the freezing point depression. The measured results obtained were converted into the ideal osmolality using the calibration equation shown in the Experimental section. In fact, the 1st calibration equation was used for sodium chloride while the 2nd one for potassium chloride, respectively. The reason was that calibration should be repeated at any time of change of measuring vessel. This was true for the second substance.

The results of osmolality values are summarized in Tables (3) and (4). The relationship between molality m (mol/kg) and osmolalitym_{os} (mOsmol/kg), similarly to molality-density connection, follows a simple proportion that can be seen on Figures (3) and (4). No deviation from theory was observed.

From those results, again, it was possible to calculate the equations relating molality to osmolality for both sodium chloride and potassium chloride. For sodium chloride, the relationship between molality m (mol/kg) and osmolality m_{os}(mOsmol/kg) could be described by equation with the coefficient of determination $R^2 = 0.9981$:

$$m_{os} = 1831.6 \cdot m + 1.5273 \quad (15)$$

Similar equation with coefficient of determination $R^2 = 0.9997$, was detected for potassium chloride:

$$m_{os} = 1867.1 \cdot m + 1.1727 \quad (16)$$

By using the equations it is possible to calculate and later see on the graphs the ideal osmolality values, for any molality value within the range 0.01mol/kg to 1.00 mol/kg.

Conversion molality to molarity

In order to convert molality to molarity, first, the volume V (ml) of the solution had to be calculated, using the experimentally estimated density and thenmolality m (mol/kg) was converted to molarity c (mol/l) using the equation (9).

The results are presented in Table 5 and Table 6.It could be seen that the differences between the values of concentration are small for very diluted solutions and could be neglected but they increase when concentration increases.

From the practical point of view, preparation of solutions in molality way is easierbecausethe temperature calibration is not required. The conversion of molality to molarity is necessary to express theoretical osmolarity (see below).

Estimation of the partial specific volume and molal volume of substance

Some of the additional information that were alsoestimated were the partial specific volume V_g (ml/g) and molal volume V_{mol} (ml/mol) from the equations (10) and (11), respectively.Those values can be seen analytically in Table 5 and Table 6. The influence of molality on the V_g and V_{mol}values is shown in Figures 5 - 8. It could be seen that the values vary around the average value from the concentration of approximately 0.03 mol/kg up to 1.00 mol/kg. In Figures, therefore, the average value is signed by a line. In opposite, the values of V_g and V_{mol} decreasewhen concentration reaches the infinite dilution, i.eat very low concentrations of 0.01 mol/kg and 0.02 mol/kg.

The average V_g and V_{mol}values for sodium chloride are 0.29 ml/g and 16.80 ml/mol, respectively, while the average values for potassium chloride are 0.36 ml/g and 26.80 ml/mol, respectively. Similar results were previously presented by Storkova (2014) with the average values of V_g and V_{mol}0.30 ml/g and 17 ml/mol for sodium chloride and 0.37 ml/g and 27 ml/mol for potassium chloride, respectively. The molal volumes of both substances at infinite dilution of 16.63 ml/mol for NaCl and 26.74 ml/mol for KCl were estimated (Streng et al. 1978). The values of V_{mol} were close to the experimental results of this thesis.

Estimation of molal osmotic coefficient Φ_m

Molal osmotic coefficient Φ_m is one more value that was also possible to be calculated from the previously estimated osmolality m_{os} according to the equation (12). That value should be close to 1 (but not over 1) and it decreases as the concentration increases. Those results can be found on Table 7, Table 8 and on

Figure 9 and Figure 10.The value of Φ_m decreases if concentration increases (USP 35/NF 30 2011; Ph. Eur. 8.3). The results noted complied to the theory.

Approximation of osmolarity

To expect the osmotic effect of parenteral infusions, osmolarity value is required. Unfortunately, osmolarity cannot be measured. To express the values of osmolarity c_{os} (osmol/l), the calculation is required. It is possible to calculate the osmolarity values from 3 different equations (USP 35/NF 30 2011).

One is the calculation of the ***theoretical*** osmolarity (Eq. 4) from the molarity of the solution. Thus, the conversion from molality to molarity, which requires measurement of the solution density, is necessary. The other onesare calculation of osmolarity from the exeprimentally determined $\boldsymbol{V_g}$(Eq. 5) and/or "***water contentmethod***" (Eq. 6). The method of approximation is described above in Theoretical section (5.2.2) and Experimental section.The results can be found in Table 7 and Table 8 and on Figure 11 and Figure 12.Although the differences between measured osmolality and the estimated osmolarity are very low, below concentration limit of 0.1 mol/kg, they increase when concentration approaches 1 mol/kg. The theoretical approximation is the least accurate as could be seen in Figures 11 and 12. It can only describe an ideal behaviour of a substance where its particles don not interact with each other. According to the results from the three different methods, the most precise way to determine osmolarity is by using the experimentally determined density and osmolality followed by employing the partial specific volume Vg and/or the "water content".

9 Conclusions

From the results of this thesis the following conclusions were drawn:

1. The relationship between molality and density as well as between molality and osmolality follow the simple proportion in which both variables increase if concentration raises.
2. The average values of V_g and $V_{mol}$for sodium chloride of 0.29 ml/g and 16.80 ml/mol, respectively, and 0.36 ml/g and 26.84 ml/mol for potassium chloride, respectively, were experimentally detected.
3. To convert molality to molarity,the density of the solution is necessary to be determined.
4. The molal osmotic coefficient Φ_m decreases steadily when molality increases.
5. To estimate osmolarity with the highest accuracy, the method using the partial specific volume Vg and/or the "water content" can be recommended for soduim chloride and potassium chloride.
6. To convert osmolality to osmolarity precisely, the density of the solution and osmolality measurements are necessary values that need to be determined.

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